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MATHEMATICAL MODELING OF MITOSIS

Summary of the thesis

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2013

Introduction

Appearance of eukaryotes was a milestone of capital importance on the road of evolution leading up to the amazing diversity of present life. Their revolutionary breakthrough was the development of novel mechanisms which allow the reliable and fast replication of orders of magnitude more genetic material than the amount found in prokaryotes. For the sake of this capability strict separation of DNA replication (S phase) and of sister chromatid segregation (mitosis, M phase) was essential.

Alternating scheduling of S and M phases is a further requirement of the eukaryotic cell cycle. Alternation of the activity of a (or in higher eukaryotes more) cyclin-dependent protein kinase-cyclin complex (CDK) between low and high values ensures this timing. CDK activity is close to zero in the G1 phase of the cell cycle. It starts to rise at the onset of DNA replication, peaks in mitosis and falls sharply at the end of mitosis. One of the central questions of cell cycle research is how transition between these two stable states of different CDK activity happens. *The molecular network regulating the transition from the state of high CDK activity to the state of low CDK activity – i.e. the completion of mitosis – was in the focus of my PhD work.* Creation and investigation of mathematical models based on the laws of biochemical reaction kinetics were my chosen tools to achieve this goal.

Background

Cell cycle is defined as all the events happening between two subsequent cell divisions. The eukaryotic cell cycle can be divided into four phases. Two of those fundamentally affects the genetic material of the cell: DNA replication (S phase) and mitosis (M phase) – when separation of sister chromatids happens. Two gaps separate these phases: G1 follows mitosis and G2 follows DNA replication. G1, S, G2 and M phases always occur in this order. Cell division happens after mitosis. The mitosis itself can be divided into several phases, which are in strict sequential order: prophase, prometaphase, metaphase, anaphase, and telophase. Mitotic phases up to metaphase are jointly called *early mitosis*, in order to distinguish those from the *completion of mitosis*¹.

Viability of the cell depends on two essential requirements: DNA replication and mitosis must be scheduled following an alternating pattern ('alternation problem'), and any of these processes must happen only if the other one has been finished

1 Morgan D. O., (2007) *The cell cycle: principles of control*. New Science Press in association with Oxford University Press; Sinauer Associates, London: Sunderland, Mass.

2 BACKGROUND

(‘completion problem’). Checkpoints ensure the satisfaction of the completion problem and two step initiation of DNA replication ensures the satisfaction of the alternation problem.

Checkpoints acting at the G1-S, the G2-M and the metaphase/anaphase transitions are mechanisms ensuring cell cycle progress only in case of the joint existence of several prerequisites. Regarding to mitosis research *spindle* (assembly) *checkpoint* (or mitotic checkpoint) operating at the boundary of metaphase and anaphase has crucial importance, since it ensures that anaphase – i.e. the segregation of sister chromatids – starts only after all of the chromosomes are aligned on the metaphase plate.

The mechanism responsible for the alternating scheduling of DNA replication and mitosis prevents re-replication of DNA before the successful segregation of sister chromatids. In essence, DNA replication is based on two dependent prerequisites, one of those coupled to the completion of anaphase. Activity of CDK (heterodimer of a *cyclin-dependent protein kinase* and a *cyclin* subunit; it can be considered as the main regulator of cell cycle) – which appears at the onset of DNA replication and peaks in mitosis – must be eliminated before the beginning of a new S phase. Since CDK activity is self-supporting, an other signal is required to abolish it. This molecular signal is the appearance of APC activity. The APC (*anaphase-promoting complex*) is activated in the anaphase of mitosis and has two main roles: it initiates the segregation of sister chromatids and destroys CDK activity. Since these two processes are initiated by the same signal, elimination of CDK activity in deed happens only after the successful segregation of sister chromatids, i.e. after finishing anaphase.

Complementary alternation of CDK and APC activity is one of the most fundamental characteristics of the eukaryotic cell cycle. Both of these activities are self-supporting and mutually exclusive: in G1 phase CDK activity is low and APC activity is high, in S-G2-M phases the reverse case stands. Hence one of the central questions of cell cycle research is how the cell switches between states of low and high CDK activity². In order to answer this question, regulation of CDK and APC must be studied.

Cyclin-dependent protein kinases (Cdks) are catalytic subunits of the CDK complex. Cdk level can be considered constant. Their regulatory partners are cyclins, which level shows periodic oscillations throughout the cell cycle. Only the Cdk:cyclin heterodimer has catalytic activity. MPF (*M-phase-promoting factor*) – which is the complex of mitotic cyclins and Cdk – has outstanding importance. CDK activity is regulated through three mechanisms: (1) cyclin synthesis and degradation, (2) activating and inhibitory phosphorylation and dephosphorylation of Cdk, and (3)

2 Nasmyth K. (1996) At the heart of the budding yeast cell cycle. *Trends Genet* 12, 405-412.

inhibition of CDK by cyclin-dependent kinase inhibitors (CKIs). CKIs act in G1 phase. Cdk-activating kinases (CAKs) activate Cdk by phosphorylation, independently of cell cycle position. Inhibitory phosphorylation of Cdk occur mainly through S and G2 phases by the Wee1 kinase. The inhibitory phosphate group of Cdk is removed by phosphatase Cdc25. Cyclins are synthesized during S–G2–M phases. Their degradation is initiated by the APC ubiquitin ligase complex. To this end association of activator subunits to the APC core – which itself is a huge heteromultimer complex – is needed. Cdc20 and Cdh1 are the two most important APC activators. APCP:Cdc20³ complex is activated during the anaphase of mitosis, while APC:Cdh1 is active from mitotic exit till the end of G1. At the metaphase/anaphase transition APCP:Cdc20 initiates the degradation of cohesin proteins – which hold sister chromatids together – as well. To this end it ubiquitinates securin, the inhibitor of separase which in turn is responsible for the degradation of cohesins.

Not only APC regulates the activity of MPF by the regulation of cyclin level, but many reverse connections were observed, too. While phosphorylation of the APC core by MPF facilitates the binding of Cdc20, it does not affect the binding of Cdh1. *Delayed activation of the APCP:Cdc20 complex (compared to MPF) has crucial importance in the regulation of mitosis. Neither the cause of this delay nor the role of Cdc20 phosphorylation in the regulation of APCP:Cdc20 is fully elucidated.* Kramer *et al.*⁴ found no effect of MPF on Cdc20, while result of Yudkovsky *et al.*⁵ suggest that *MPF inhibits Cdc20 by phosphorylation*. Activation of APCP:Cdc20 can also be prevented by protein Mad2, which forms an inactive complex with Cdc20 on activation of the spindle checkpoint. Since Cdh1 is inactivated by the inhibitory phosphorylation of MPF, activity of the counteracting phosphatase Cdc14 (in budding yeast) is also required to activate APC:Cdh1. But Cdc14 is kept in an inactive complex with Net1 in the nucleolus from G1 till early mitosis, so its activation is also required for mitotic exit. Two regulatory pathways are responsible in budding yeast for the activation of Cdc14. While FEAR (Cdc fourteen early anaphase release) ensures partial activation of Cdc14 during early anaphase, MEN (*mitotic exit network*) accomplishes this process from late anaphase. Since multiple components of MEN are regulated by MPF, regulation loops involved in mitotic exit are highly

3 APCP denotes the phosphorylated form of the APC core.

4 Kramer E. R., Scheuringer N., Podtelejnikov A. V., Mann M. and Peters J. M. (2000) Mitotic regulation of the APC activator proteins CDC20 and CDH1. *Mol Biol Cell* 11, 1555-1569.

5 Yudkovsky Y., Shteinberg M., Listovsky T., Brandeis M. and Hershko A. (2000) Phosphorylation of Cdc20/fizzy negatively regulates the mammalian cyclosome/APC in the mitotic checkpoint. *Biochem Biophys Res Commun* 271, 299-304.

complicated. Mathematical models provide valuable help for their understanding.

One possible strategy of modeling is the progression from simpler towards more complicated models. During the early embryonic cycles of the African clawed frog (*Xenopus laevis*) only concentration changes of cyclin B ‘drives’ the oscillation of MPF, other regulatory mechanisms do not operate. Neither Cdh1, nor CKIs are present, cyclin degradation depends solely on APCP:Cdc20. Presence of inhibitory phosphorylation of Cdk by Wee1 is not fully clarified. Although the spindle checkpoint is inactive, cycling extract of frog eggs can be blocked in metaphase by the addition of big amount of Mad2. As a consequence of these facts, many simple model of the embryonic cycles of *Xenopus laevis* were developed. These models usually handled the delay in APCP:Cdc20 activation by assuming an experimentally not well supported intermediary enzyme (IE)⁶. *Thus my first goal was the development of a cell cycle model, which does not assume any IE, and which takes recent findings on Cdc20 phosphorylation by MPF into account. Among my further goals was the investigation the role of key enzymes (like Cdc14) and regulatory pathways (like FEAR and MEN) of mitosis, which were not described by any former model.*

On mitotic exit several ‘executor’ proteins (EPs) are activated, e.g. phosphatases which remove the inhibitory phosphate group placed on many proteins by MPF. Since at this point of the cell cycle MPF activity drops from high to low level, assumption the role of MPF in the activation both of these proteins and their transcription factors (TFs) seems logical. If this assumption is right, MPF, the TF and the EP form a *feed-forward loop* (FFL)⁷. Three interactions are observable in a FFL. The first – which is called the ‘short arm’ of the FFL – is phosphorylation of EP by MPF. The second is phosphorylation of TF by MPF, and the third is the effect of TF on EP. The latter two together forms the ‘long arm’ of the FFL. All three interactions can be activating (+) or inhibitory (-). Although function of FFLs in cell cycle regulation was studied in some specific cases, their general role in mitotic exit is not elucidated. *Thus creation a model to investigate FLLs of (++/-) and (--/-) network topology as potentially important regulatory motifs of mitotic exit was among my goals.*

One of the leading contemporary causes of death, cancer can to a certain extent be attributed to the disorders of cell cycle regulation. Because of this, one segment of

6 Tyson J. J. and Novak B. (2001) Regulation of the eukaryotic cell cycle: molecular antagonism, hysteresis, and irreversible transitions. *J Theor Biol* 210, 249-263.

7 Mangan S. and Alon U. (2003) Structure and function of the feed-forward loop network motif. *Proc Natl Acad Sci USA* 100, 11980-11985.

chemotherapeutic agents used in the treatment of cancer (e.g docetaxel, paclitaxel) inhibits uncontrolled cell divisions by activating the spindle checkpoint. Unfortunately, cancer can not be cured so easily since the activity of the *chemoimmunity system*⁸. Components of this system – nuclear receptors, metabolic enzymes and multidrug transporters (typically ABC transporters) – act in a regulated manner resembling the operation of the immune system to defend the cell from the adverse effects of xenobiotics, hence also from chemotherapeutic agents. *Thus a further goal of mine was the creation of a model and a database which help to understand the chemoimmunity system and particularly ABC transporters.*

Methods

The complexity of the molecular network regulating mitosis makes its understanding solely based on intuitions insufficient. Because of this, I have chosen the creation and analysis of mathematical models based on laws of biochemical reaction kinetics as my tool⁹. Input information of models came from wet lab experiments. I did not do any of those experiments, rather used results of experimentalists published in papers and biological databases. As a first step, ‘*wiring diagram*’ of interacting molecules was created as a synthesis of input information. Based on the wiring diagram and using the laws of biochemical reaction kinetics equations were formulated to describe the change of concentration of components with time. These equations (which mostly are ordinary, nonlinear differential equations), their parameters and the related initial conditions together form the *mathematical model*. *Time course simulations* were conducted by numerically solving the set of equations. Concentration profiles changing with time are the results of these simulations. Version 6.10 of the open source, freely available software XPPAUT¹⁰ was used for the numerical integration of the equations. *Phase plane and bifurcation analysis – two methods of the theory of dynamical systems* – were applied to further investigate the dynamical properties of the models. Outcomes got using all of these methods were subsequently compared to the results of laboratory experiments served as the input of modeling. If experimental and modeling results were in agreement, I assumed that the model adequately grasps the essence of the

8 Sarkadi B., Homolya L., Szakács G. and Váradi A. (2006) Human multidrug resistance ABCB and ABCG transporters: participation in a chemoimmunity defense system. *Physiol Rev* 86, 1179-1236.

9 Tyson J. J., Chen K. and Novak B. (2001) Network dynamics and cell physiology. *Nat Rev Mol Cell Biol* 2, 908-916.

10 XPPAUT is available at <http://www.math.pitt.edu/~bard/xpp/xpp.html>.

studied biological phenomenon. In the opposite case – when altering model parameters was insufficient to get a satisfactory agreement between experimental and modeling results – I assumed that the wiring diagram does not adequately reflect the real biological relations, so it must be modified. Simulations were redone after the alteration of the wiring diagram and affected equations. This process was repeated until experimental and modeling results showed satisfactory similarity¹¹.

Results

Both early mitosis and completion of mitosis were studied in detail. First, the regulation of APCP:Cdc20 (i.e. particularly the events of early mitosis) was investigated by modeling the early embryonic cycles of the African clawed frog (*Xenopus laevis*). Next, by using a mitosis model (which contains APC:Cdh1, the FEAR and MEN pathways, too) of budding yeast (*Saccharomyces cerevisiae*) I focused on the study of completion of mitosis. Afterwards, the role of feed-forward loops in the completion of mitosis was analyzed. Finally, I created a model of the chemoimmunity system and participated in the development of a mutation database of ABC transporters.

Regulation of APCP:Cdc20 in Xenopus laevis

Since its simplicity, modeling the embryonic cycles of *Xenopus laevis* has been chosen to investigate the regulation of APCP:Cdc20. First, the intermediary enzyme was removed from the 2001 model¹² created by Tyson and Novak. A novel – and hopefully biologically more adequate – mechanism based on MPF's different effects on the APC core and Cdc20 has been held responsible for the delayed activation of APCP:Cdc20. Not only the effect, but the rate of phosphorylation of the APC core and Cdc20 is also different (Fig. 1). Hence APCP:Cdc20 – which ubiquitinates cyclin B (part of MPF) – is under dual regulation by MPF: by phosphorylation MPF activates the APC core but inactivates Cdc20¹³. Since the inactive forms have decreased affinity to each other, in the model for the sake of simplicity only APCP and Cdc20 can associate and form the APCP:Cdc20 complex, which has ubiquitin ligase activity.

11 Tyson J. J., Csikasz-Nagy A. and Novak B. (2002) The dynamics of cell cycle regulation. *Bioessays* 24, 1095-1109.

12 Tyson J. J. and Novak B. (2001) Regulation of the eukaryotic cell cycle: molecular antagonism, hysteresis, and irreversible transitions. *J Theor Biol* 210, 249-263.

13 Cdc20P denotes the phosphorylated form of Cdc20.

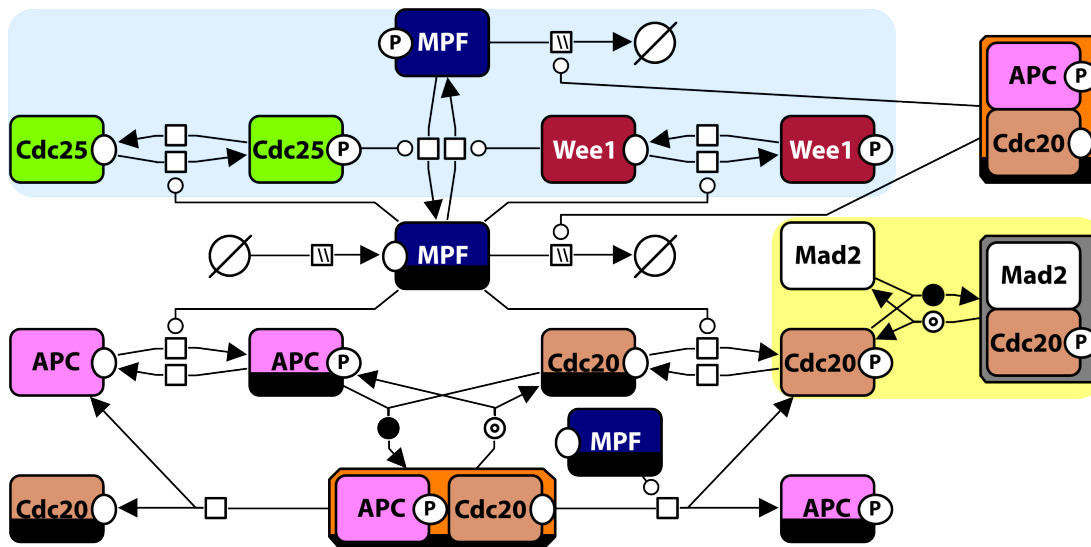


Figure 1. Wiring diagram of early embryonic cycle models of *Xenopus laevis*. Yellow background: spindle checkpoint module. Blue background: MPF phosphorylation module.

Time course simulation of the model results in stable limit cycle oscillations, which can be attributed to the different rate of APC and Cdc20 phosphorylation. Both phosphorylation and dephosphorylation of Cdc20 are faster compared to APC phosphorylation and dephosphorylation. Therefore MPF first act as an inhibitor, and later as an activator of APCP:Cdc20. This creates a time window of 10-20 minutes when MPF activity is high and events of early mitosis can be completed. Eventually, rising concentration of APCP:Cdc20 results in accelerating cyclin B degradation, hence in the inactivation of MPF and finally exit from mitosis.

As a next step the model was complemented by Mad2 and a simplified description of the spindle checkpoint. I assumed that Mad2 acts by forming an inactive complex with phosphorylated Cdc20P when the checkpoint is activated, and this way blocks progression of the cell cycle (Fig. 1). Using time course simulations experiment of Li *et al.*¹⁴ was successfully modeled. In that experiment addition of recombinant Mad2 to the cycling extract of frog eggs activated the spindle checkpoint. In order to conduct phase plane analysis the model was transformed to contain only two dynamical variables. It was shown, that the system modeling the cycling embryo extract has a single stable attractor which is a limit cycle. On activation of the spindle checkpoint this attractor disappears and a stable node becomes the single stable state of the system. High MPF activity characteristic to this point prevents the accumulation of dephosphorylated Cdc20, thus APCP:Cdc20 can

14 Li Y., Gorbea C., Mahaffey D., Rechsteiner M. and Benezra R. (1997) MAD2 associates with the cyclosome/anaphase-promoting complex and inhibits its activity. *Proc Natl Acad Sci USA* 94, 12431-12436.

not be activated and cell is blocked in mitosis. Bifurcation analysis of the system revealed that activation of the spindle checkpoint probably happens via a saddle-loop infinite period bifurcation of the system.

Since according to recent experiments inhibitory phosphorylation of MPF may also plays a role in the regulation of early embryonic cycles of *Xenopus laevis*, the model has been extended with a MPF phosphorylation module containing the kinase Wee1 and the phosphatase Cdc25 (Fig. 1). Although some former *Xenopus laevis* models already contained this module^{15,16}, the description applied did not completely satisfy an application criterion of Michaelis–Menten kinetics, namely that the amount of enzyme must be negligible compared to the amount of its substrate. The complication is due to the fact, that Wee1 and Cdc25 are enzymes in certain reactions, but substrates in others. Because of this, in opposite to the 1993 model, concentration change of Wee1 and Cdc25 was not described by the Goldbeter–Koshland function. Enzyme kinetics was only applied to describe the dephosphorylation of Wee1 and Cdc25 (by unspecified phosphatases), all other reactions were described using mass action kinetics. Hence equations satisfy all application criteria of Michaelis–Menten kinetics. Time course simulation and bifurcation analysis were performed using the extended model, which gave similar, but slightly different results than those obtained by the previous versions of the model. The length of G1 and S phases was increased, just like the period and amplitude of MPF oscillation. Mitotic exit this time is related to a saddle-node infinite period bifurcation of the system.

Completion of mitosis in budding yeast

To study the completion of mitosis I searched for an organism which has more complex regulatory pathways than those observable during the embryonic cycles of *Xenopus laevis*. I chose budding yeast, which contains phosphatases PP2A and Cdc14, APC:Cdh1 (which is activated by the latter), and the FEAR and MEN pathways, which play role in the mechanism of activation (Fig. 2). Queralt *et al.* published a model (2006)¹⁷ which they used to perform *time course simulations* of

15 Novak B. and Tyson J. J. (1993) Numerical analysis of a comprehensive model of M-phase control in *Xenopus* oocyte extracts and intact embryos. *J Cell Sci* 106 (Pt 4), 1153-1168.

16 Marlovits G., Tyson C. J., Novak B. and Tyson J. J. (1998) Modeling M-phase control in *Xenopus* oocyte extracts: the surveillance mechanism for unreplicated DNA. *Biophys Chem* 72, 169-184.

17 Queralt E., Lehane C., Novak B. and Uhlmann F. (2006) Downregulation of PP2A(Cdc55) phosphatase by separase initiates mitotic exit in budding yeast. *Cell* 125, 719-732.

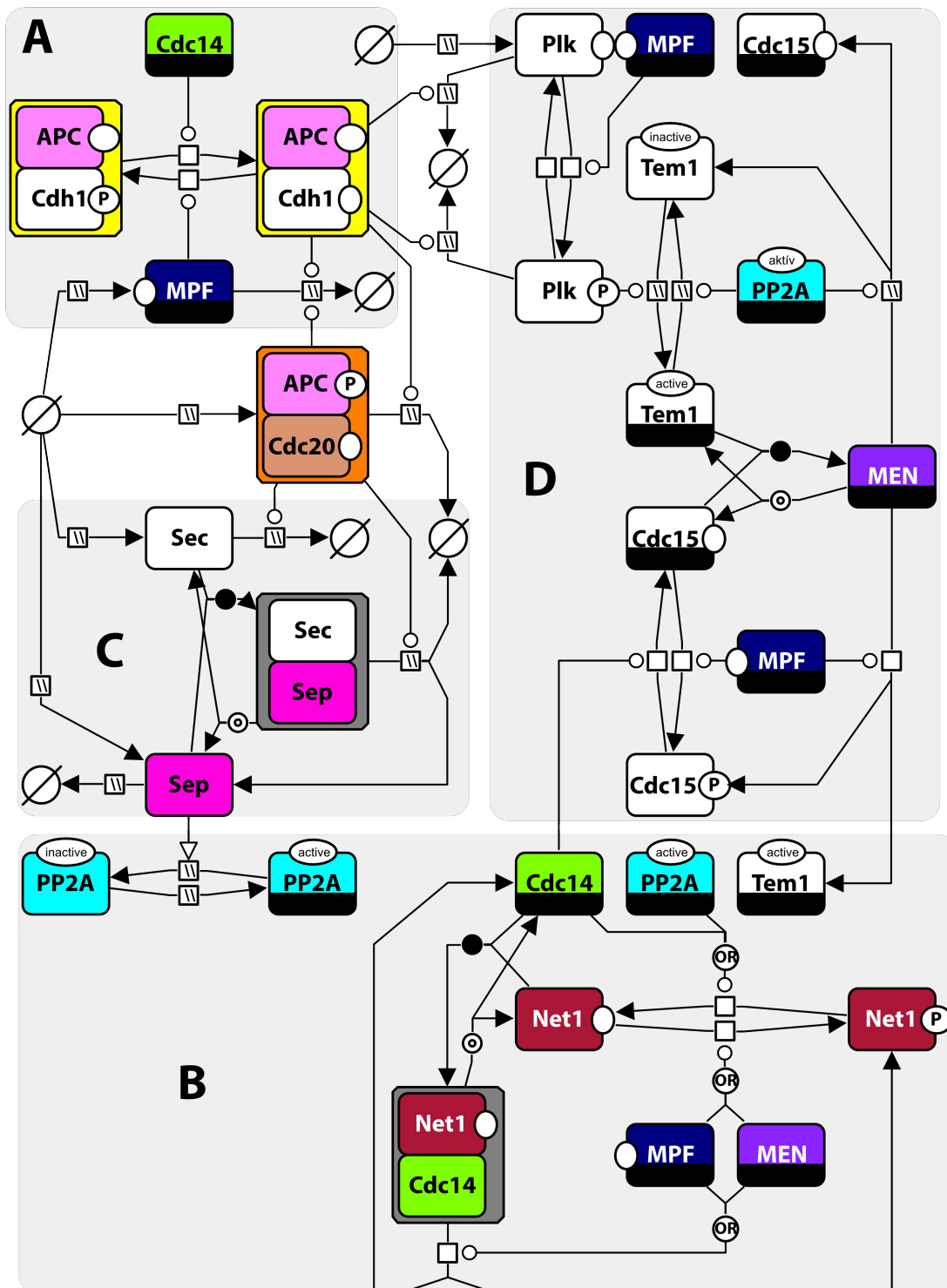


Figure 2. Wiring diagram of models describing completion of mitosis in budding yeast. Module A: antagonism of MPF and APC:Cdh1. Module B: Cdc14 regulation by Net1. Module C: regulation of separase by securin. Module D: the MEN pathway.

several mutants defective in the regulation of mitosis. This model was selected as the starting point of my work. I transformed it to contain only *two dynamical variables* and used the derived model for *phase plane analysis*.

This model does not contain the MPF phosphorylation module, since it was designed to not to describe the whole cell cycle, but only the events from anaphase onset (state of high MPF activity) till the *completion of mitosis* (state of low MPF activity). On the other hand several components of the FEAR and MEN pathways were gradually introduced to the model. This way models from simpler towards more complicated were developed. In each step two, closely related models were developed. Models of either series – which use differential equations to describe the concentration change of components with time – were used to carry out time course simulations. Models of the other series were created from the corresponding model of the first series using steady state assumptions. These ‘two-dimensional’ models were used to analyze the dynamical behavior of the system on the MPF–Cdc14 phase plane. Results of time course simulations and phase portraits – where possible – were compared to experimental results collected from the literature.

Phase plane analysis showed that a saddle-node bifurcation on the MPF–Cdc14 phase plane is required for mitotic exit. To this end Cdc14 activity has to rise above a certain threshold. To investigate the role of the FEAR pathway, outcome of wet lab experiments were analyzed by phase plane analysis of MEN mutants. I showed that although ectopic overexpression of separase or activation of APCP:Cdc20 causes – in agreement with the experimental results stable or transient – Cdc14 release, but this is under the threshold required for mitotic exit, thus the cell cycle is blocked in mitosis.

Afterwards I focused on the role of the MEN pathway. Phase plane analysis showed that mitotic exit depends on an inhibitory and an activation MPF threshold. The inhibitory threshold is related to the MPF driven inactivation of protein kinase Cdc15, a downstream MEN component. The activation threshold in turn is related to the MPF driven activation of GTPase Tem1, an upstream MEN component. Mitotic exit happens only when MPF activity is below the inhibitory, but at the same time above the activation threshold.

Further analysis revealed two bistable switches of the underlying regulatory network. Exit from mitosis happens by the subsequent irreversible transition of these switches (in a certain order), and degradation of both substrates of APCP:Cdc20 is essential for the process in wild type cells. First, degradation of securin in a FEAR-regulated manner ‘turns on’ the positive feedback loop between Cdc14 and MEN. This activation of the MEN pathway leads to the full release of Cdc14 from the nucleolus. Second, cyclin degradation results in the decrease of the Cdc14 threshold

of the MPF nullcline required for mitotic exit. Activation of Cdc14 and decreasing Cdc14 threshold together cause the irreversible transition of the other bistable switch: MPF gets completely inactivated and activity of APC:Cdh1 rises. The coordinated operation of these two bistable switches ensures the robust, reliable regulation of mitotic exit.

Role of feed-forward loops in the completion of mitosis

In order to study the role of feed-forward loops in the regulation of the expression wave observable in late mitosis and G1 a model consisting of a simple cell cycle 'engine', two TFs and two EPs was constructed. These equations describe the (+ +/ -) and (- -/ -) type feed-forward loops, considering the different time scale of phosphorylation and protein synthesis. Time course simulation shows transient activation of EPs, which happens only at mitotic exit. Since the inhibitory effect of MPF is solely based on EP phosphorylation through the short arm of the FFL, this effect – which acts on the second level scale – vanishes when MPF activity sharply drops at the end of mitosis. Contrary, since protein synthesis is also involved in the activation of EPs through the long arm of the FFL, this effect operates on the minute level scale and fades out slower than the inhibitory effect. As a conclusion, (+ +/ -) and (- -/ -) type feed-forward loops are generally applicable network motifs of cell cycle regulation which can ensure transient activation of executor proteins at the completion of mitosis.

Investigation the relation of mitosis to the chemoimmunity system

To better understand the effect of chemotherapeutic agents on cell cycle, I created a reaction kinetic model of the chemoimmunity system and took part in the development of a novel database of ABC transporter mutations. The model describes the interaction between a single compound and two ABC transporters, one cytochrome oxidase and one glutathione transferase. The regulation of these enzymes are modeled by transcriptional and translational feed-forward and feedback loops. By taking into account the toxicity of the compound and its metabolites, analysis of cytotoxic effects of drugs is also possible.

The first step in development of the database of ABC transporter mutations was the creation of MutMiner¹⁸, a data mining framework capable for the detection of protein mutations in PDF files with high specificity and sensitivity. Thereafter

18 MutMiner is available at <http://mutminer.hegelab.org>.

MutMiner was used to collect mutations of members of the ABC subfamilies A, B, C, D and G from publications downloaded through the PubMed database. 80% of mutations also available in other databases was found, and additionally many mutations not present in these databases were identified. All of these mutations were presented on-line at <http://abcmutations.hegelab.org>, in the freely available ABCMdb database. Using the web interface mutations can be searched, browsed, displayed on the three dimensional structure of proteins, and compared to amino acids found in homologous positions of other ABC proteins.

New scientific results

1. A new reaction kinetic model of the early embryonic cycles of the African clawed frog (*Xenopus laevis*) was developed. The intermediary enzyme (IE) – used in former models due to technical reasons, but never supported by satisfying biological evidence – was omitted from the model. At the same time the model contains the first mathematical description of the inhibitory effect of MPF phosphorylation on Cdc20. Time delay in the activation of APCP:Cdc20 can be explained by the two opposing (and on different time scales acting) effects of MPF on the APC core and Cdc20, which in turn can result in limit cycle oscillations well usable for the description of early embryonic cycles (Ciliberto *et al.*, 2005).
2. The model of the early embryonic cycles of *Xenopus laevis* was extended with a simple description of the spindle checkpoint. A simplified model containing only two dynamical variables was created, too. Using phase plane analysis I showed that the only stable attractor of the system modeling the cycling egg extract is limit cycle oscillation. On addition of recombinant Mad2 to the extract this attractor disappears and a stable node of high MPF and APCP level becomes the single stable state of the system. This transition implies mitotic block. Bifurcation analysis showed that activation of the spindle checkpoint probably can be attributed to a saddle-loop infinite period or a saddle-node infinite period bifurcation of the system (Ciliberto *et al.*, 2005).
3. The model of the early embryonic cycles of *Xenopus laevis* was further extended with the description of inhibitory MPF phosphorylation by Wee1. Contrary to a formulation widely used in former models – but does not completely fulfill an application criterion of Michaelis–Menten kinetics – I applied such a combination of enzyme and mass action kinetics, which satisfy the mentioned criterion (Ciliberto *et al.*, 2005).
4. I created a simplified budding yeast mitosis model containing only two

dynamical variables based on the model of Queralt *et al.* (2006). Phase plane analysis showed that a saddle-node bifurcation on the MPF–Cdc14 phase plane is required for mitotic exit. To this end Cdc14 activity has to rise above a certain threshold. Existence of an inhibitory and an activation MPF threshold was revealed, too. Mitotic exit happens only when MPF activity is between these thresholds (Tóth *et al.*, 2007).

5. Further analysis of the simplified budding yeast mitosis model disclosed two bistable switches of the underlying regulatory network. Exit from mitosis happens by the subsequent irreversible transition of these switches (in a certain order), and degradation of both substrates of APCP:Cdc20 is essential for the process in wild type cells. The coordinated operation of these bistable switches ensures the robust, reliable regulation of mitotic exit (Tóth *et al.*, 2007).
6. A general model of the (+ +/ -) and (- -/ -) type feed-forward loops was developed to study the role of feed-forward loops in the regulation of mitotic exit. Time course simulation showed that these feed-forward loops are generally applicable network motifs of cell cycle regulation which can ensure transient activation of executor proteins at the completion of mitosis (Csikász-Nagy *et al.*, 2009).
7. I took part in the development of a novel database of ABC transporter mutations. ABCMdb provides easy and free access not only to most of the mutations available from other databases, but also to many mutations not available from other databases (Gyimesi *et al.*, 2013).

Scope of research

During my PhD work I created models of early mitosis and the completion of mitosis, which are more detailed, presumably biologically more adequate and in many ways mathematically more correct than former models. Deeper understanding (from a dynamical point of view) of molecular networks regulating mitosis is the key benefit of these models, which has opinion-leading power regarding to future research. Modeling techniques used to replace the hypothetical intermediary enzyme and the former, not completely valid application of Michaelis–Menten kinetics can be used for the improvement of existing models and during the development of new ones. My results may give inspiration for further research. For example, incorporation of models here applied specifically for the investigation of mitosis into general cell cycle models could be a great opportunity for the more realistic modeling of the whole cell cycle. Likewise combining the model of the chemoimmunity system

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with cell cycle models could be beneficial during the development of chemotherapeutic agents.

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Poster presentations

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- TÓTH A., QUERALT E., UHLMANN F. AND NOVÁK B. Mitotic exit in two dimensions. *Computational Cell Biology Meeting*, Cold Spring Harbor Laboratory, Cold Spring Harbor, USA, 2007. March 6-9.

Oral presentation

- TÓTH A. A sztereocíliumok hossz-szabályozásának modellezése (Modeling length regulation of stereocilia). *Conference of PhD Students*, Budapest University of Technology and Economics, Faculty of Chemical Technology and Biotechnology, 2006. February 7. (In Hungarian)

